Chapter 8

Summary, general discussion and future perspectives

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SUMMARY

Worldwide, the prevalence of diabetes is on the rise, with more than 400 million people affected today[1] and this number expected to exceed 550 million by 2030.[2] People with type 2 diabetes are at an increased risk of cardiovascular disease (CVD) and premature mortality. There is therefore a considerable need to reduce the CV risk of those living with diabetes. This increased CV risk is the result of the interaction of various risk factors, only one of which is hyperglycemia. Given the heterogeneity of the disease there are no simple solutions to reduce the impact of diabetes and the associated CV risk. However, for assessing CV risk and CV mechanisms in people with type 2 diabetes novel imaging modalities and innovative techniques may be useful. Vascular imaging brings to light surrogate markers for atherosclerosis, which is the underlying process of CVD. Vascular imaging can reveal arterial ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake as a marker of CV risk, thereby enhancing our insight into the development of CVD. Vascular imaging can also be used to monitor treatment effects. Besides vascular imaging, other imaging modalities can help to reveal CV risk markers like the extent of abdominal visceral adipose tissue. Furthermore, innovative techniques such as pulse wave velocity (PWV) may be also useful. Part I of this thesis presents an overview of different aspects of CV risk imaging. Part II of the thesis focuses on the clinical application of vascular imaging and discusses other CV risk markers in people with early type 2 diabetes. Part II also discusses the treatment effects of linagliptin (DPP-4 inhibitor) on arterial ^{18}F-FDG uptake and PWV. **Chapter 1** provides the general introduction to the thesis and outlines its aims.

PART I

Chapter 2 reviews imaging modalities, including different imaging agents used to identify pathophysiological processes occurring within the high-risk atherosclerotic plaque. Various imaging modalities such as nuclear imaging, bio-optical imaging, bioluminescence, fluorescence and multispectral optoacoustic tomography are available to identify pathophysiological processes occurring within the high-risk plaque. However, not all of these imaging modalities can be used *in vivo* as they can be hampered by such hindrances as limited penetration depth of the fluorescent signal or the need to transfect cells with the luciferase gene.

Up to now, the best validated and most frequently used agent for vascular imaging is the radiopharmaceutical ^{18}F-fluorodeoxyglucose (^{18}F-FDG). ^{18}F-FDG is taken up by inflammatory processes associated with atherosclerosis and can be assessed using hybrid imaging systems such as positron emission tomography (PET) co-registered with computerized tomography (CT) scanning. In addition to ^{18}F-FDG, ^{18}F-sodium fluoride (^{18}F-NaF), which is incorporated

in microcalcifications, is also a promising imaging agent for assessment of atherosclerosis. In **Chapter 3** we assessed the feasibility of ^{18}F-NaF as a marker of atherosclerotic plaque vulnerability. We also investigated *ex vivo* whether ^{18}F-NaF uptake is different in symptomatic and asymptomatic human carotid plaques. In total, we included 23 carotid plaques (17 symptomatic, 6 asymptomatic) of 23 patients undergoing carotid endarterectomy. We incubated carotid plaques in ^{18}F-NaF for one hour and scanned them using a μPET and a μCT scan. Between symptomatic and asymptomatic carotid plaques the mean ^{18}F-NaF uptake did not differ. Interestingly, the ^{18}F-NaF uptake on μPET, compared with calcification visualized on μCT, showed a discordant pattern of calcification. Therefore, we concluded that the ^{18}F-NaF uptake on PET represents a different stage in the atherosclerosis process than calcification assessed with μCT. Since the ^{18}F-NaF uptake between symptomatic and asymptomatic carotid plaques was comparable, ^{18}F-NaF is probably of greatest value in the early identification and assessment of CV risk, before the occurrence of end-stage atherosclerosis calcification as assessed with CT.

Another innovative imaging technique for assessing CV risk is the quantitative assessment of abdominal adipose tissue. The presence of visceral abdominal adipose tissue (VAT) is a major contributor to CV risk.[3,4] Recently it has been suggested that inflammation of this tissue is also linked to CV risk. ^{18}F-FDG, as a glucose analog, is taken up by cells with high metabolic activity, such as cancer cells, but also by inflamed tissue. Therefore, in **Chapter 4** we describe the optimized settings for automated assessment of abdominal adipose tissue using a ^{18}F-FDG-PET/CT scan. This automated method is highly reproducible and repeatable for quantitative assessment of abdominal adipose tissue and for the measurement of ^{18}F-FDG uptake in VAT. In addition, the reproducibility and repeatability of this automated method of measuring ^{18}F-FDG uptake in VAT make it superior to the manual method.

PART II

In **Chapter 5** we present our investigation of the association between arterial stiffness, assessed as aortic pulse wave velocity (PWV), and subclinical vascular inflammation, assessed as arterial ^{18}F-FDG uptake, in people with early type 2 diabetes. PWV and arterial ^{18}F-FDG uptake are both surrogate markers of early atherosclerosis and associated with CV risk. We observed a positive association between PWV and arterial ^{18}F-FDG uptake. These data suggest that vascular inflammation is involved in arterial stiffness in early type 2 diabetes. In contrast to arterial ^{18}F-FDG uptake and PWV as markers of early atherosclerosis, calcification represents late stages of atherosclerosis. In addition, neither arterial ^{18}F-FDG uptake nor PWV were associated with arterial calcification as visualized on CT. The mechanisms leading to CVD in people with type 2 diabetes are complex and can only be partly explained by classic CV risk factors. The finding that arterial inflammation is associated with arterial stiffness,

which is a predictor of CVD and mortality in people with early type 2 diabetes, suggests that arterial inflammation is a contributor to increased CV risk in type 2 diabetes. Therefore, arterial inflammation may be an additional target for therapy to reduce CV risk in type 2 diabetes.

Dipeptidyl peptidase (DPP)-4 inhibitors are a class of oral antidiabetic agents, which may exert favorable off target CV effects, particularly since DPP-4 is well recognized to be immunologically active. In the RELEASE [off ta**R**get **E**ffects of **L**inagliptin monoth**E**rapy on **A**rterial **S**tiffness in **E**arly diabetes] study, the effect of 26 weeks of treatment with the DPP-4 inhibitor lingaliptin on arterial inflammation and arterial stiffness was investigated. The RELEASE study was a single-center, randomized, prospective, double-blind, placebo-controlled, parallel-group, phase 3 study. Participants were males and females, diagnosed with type 2 diabetes and, because they were without a history of CVD and naïve to antidiabetic treatment, referred to as early type 2 diabetes. In total 44 participants were randomized (1:1) to once daily linagliptin 5 mg or a placebo. The primary endpoint of the RELEASE trial was PWV (corrected for SBP) after 26 weeks of therapy. In **Chapter 6** we present the treatment effects of linagliptin on PWV, central systolic blood pressure (SBP) and augmentation index (AIx), as measures of arterial stiffness. After 26 weeks, PWV was significantly lower in the linagliptin group compared with the placebo group; the between-group-difference was 0.91 m/s (95% CI 0.06-1.76 m/s, P=0.035). Central SBP and AIx from baseline throughout treatment did not differ significantly between the linagliptin and placebo groups. As expected, linagliptin decreased HbA_{1c} (-0.4%; P<0.001), fasting plasma glucose (-0.7 mmol/l; P=0.002), and triglycerides (-0.49 mmol/l; P=0.019) as compared to the placebo. **Chapter 7** shows the results of treatment with linagliptin on arterial inflammation as measured by arterial ^{18}F-FDG uptake. Arterial ^{18}F-FDG uptake was quantified as the pre-scan glucose corrected maximal standardized uptake value and corrected for background activity (target-to-background ratio, TBR) and averaged for the total aortic tree ($_{mean}$TBR). At 26 weeks, the decrease in $_{mean}$TBR under linagliptin exceeded that under the placebo by 0.18 units (95% CI, 0.04 to 0.32; P=0.015). Taken together, the results of the RELEASE trial confirm the hypothesis based on previous observations, that linagliptin exerts favorable vascular effects beyond glucose-lowering in people with early type 2 diabetes and without CVD.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In part I of this thesis we aimed to acquire knowledge regarding different aspects of imaging to increase our insight into the development of CVD. In part II we aimed to gain more insight into the relationship between different CV markers in people with early type 2 diabetes, and we investigated the therapeutic effects of linagliptin on CV risk markers such as arterial stiffness and arterial inflammation.

The myth of the "high-risk" plaque

During the past decade, the CV research field has focused on the quest to identify high-risk, vulnerable, atherosclerotic plaques in people.[5] Identifying people at increased risk of an acute CV event, like myocardial infarction or stroke, has been hypothesized as a useful strategy to identify those persons who will benefit most from intensified preventative measures,[6] measures such as a more stringent treatment goal for systolic blood pressure or LDL-cholesterol, but also surgical removal of atherosclerotic plaques. Successful prevention of CVD is greatly needed to reduce the current health burden and financial costs of CVD.[6]

Pathological processes accompanied by high-risk plaques are targets for imaging, as discussed in Chapter 2.[7-9] However, the results of clinical studies using imaging modalities to improve CV risk prediction by identifying individual high-risk plaques are rather disappointing.[6,10-12] For example, the results of the large prospective clinical trial, PROSPECT [**P**roviding **R**egional **O**bservations to **S**tudy **P**redictors of **E**vents in the **C**oronary **T**ree] demonstrated that the presence of coronary atherosclerotic plaque characteristics conferred only a low risk of future myocardial infarction or sudden cardiac death.[13] Moreover, although carotid ^{18}F-FDG uptake is associated with future CV events,[14,15] the translation of ^{18}F-FDG uptake into individual CV risk assessment is doubtful. In addition, there is a substantial overlap in arterial ^{18}F-FDG uptake between healthy controls and people with increased CV risk.[16] Therefore, the optimal choice of threshold values to predict plaque rupture is unclear.

The major limitation of identifying individual high-risk plaques and claiming an independent CV risk prediction of certain plaque characteristics is that most of the plaques are present in people already at high CV risk or who already have CVD. Therefore, the high-risk plaques are themselves a feature of atherosclerotic disease, which is strongly linked with CV risk.[5] Consequently, the additional CV risk conferred by certain plaque characteristics beyond atherosclerotic disease burden is difficult to address. The possibility of predicting CV risk by means of certain plaque characteristics may also be hampered by the fact that many plaques rupture without overt clinical events; this is also known as silent plaque rupture.[17-19] Although plaque ruptures and their healing may frequently occur without symptoms, the healed plaque will, over time, lead to progressive lumen obstruction (calcification).[20-23] Calcifications are thought to be generated as a defense against progressive inflammation within the plaque. Microcalcifications are expected to present plaque progression and a high-risk plaque feature, in contrast to established calcifications which are seen as end stage products and associated with plaque stability.[23,24] Furthermore, symptoms of plaque rupture are temporary, and can therefore only be detected within a certain time frame. Taken together, the true rate of silent plaque rupture is probably much higher than that currently estimated by imaging modalities.[5] Consistent with this notion, the results presented in Chapter 3, in which ^{18}F-NaF uptake (microcalcification tracer) did not differ between symptomatic and asymptomatic carotid plaques, are not surprising. The asymptomatic carotid plaques were associated with a high degree of stenosis (>70%)

and were present in people with existing advanced atherosclerotic disease. It is therefore reasonable to assume that the asymptomatic carotid plaques are probably a result of silent plaque ruptures. Accordingly, the plaque characteristics are not different from symptomatic carotid plaques. However, ^{18}F-NaF may still be valuable as a marker of atherosclerotic plaque for early identification and risk assessment in people without advanced atherosclerotic disease. Such a clinical value of ^{18}F-NaF should be investigated in future trials.

Abdominal adipose tissue

Abdominal adipose tissue, and in particular the volume of visceral adipose tissue (VAT), is linked to CV risk.[25,26] Recently, there is a growing interest in the inflammatory state of VAT, as this may also be linked to CV risk.[27-32] In Chapter 4 we have developed and demonstrated the applicability of a semi-automated method for abdominal adipose tissue analysis by means of a ^{18}F-FDG-PET/CT scan. However, the current study does not address the question of whether the volume of VAT is indeed positively associated with the inflammatory state measured as ^{18}F-FDG uptake in VAT as hypothesized. Additional work in a larger population is required to investigate the association between the volume of VAT and the inflammatory state of VAT. Furthermore, to detect evidence for the association between ^{18}F-FDG uptake in VAT and increased CV risk, other investigations are also needed. For example, the association between ^{18}F-FDG uptake in VAT and insulin resistance, which is an independent marker of CV risk, is of interest. Additionally, the association of VAT with other CV risk markers such as PWV and arterial ^{18}F-FDG uptake, should be investigated. These findings may help us to further understand the clinical relevance of ^{18}F-FDG uptake in VAT and the connection between abdominal obesity and CV risk.

The study in Chapter 4 also included participants with a normal weight, although the analysis of abdominal adipose tissue is especially of interest in obese subjects. The volume of VAT, and probably the inflammatory state of VAT, can help to distinguish metabolically unhealthy obese people with a high CV risk and metabolically healthy obese people with a lower CV risk.[33-35] Therefore, the abdominal adipose tissue should also be investigated in obese people by use of ^{18}F-FDG-PET/CT.

RELEASE participants are predominantly obese, have early type 2 diabetes and are consequently at increased risk of developing CVD. Moreover, CV risk markers such as PWV and arterial ^{18}F-FDG uptake were assessed in the RELEASE participants. Therefore several of the research questions raised above could be investigated using these RELEASE participants.

Another question raised is whether treatment with a DPP-4 inhibitor will have an effect on VAT, in particular ^{18}F-FDG uptake in VAT. Evidence suggests that DPP-4 is an adipokine.[36] In addition, DPP-4 expression is increased in VAT when compared with SAT; DPP-4 is released by VAT; DPP-4 induces insulin resistance in adipocytes; and DPP-4 levels are positively correlated with leptin and negatively with adiponectin.[36,37] Furthermore, VAT and SAT obtained during open abdominal surgery demonstrated that DPP-4 expression in VAT was

systematically higher compared with SAT.[38] Interestingly, the same study showed that DPP-4 expression in adipose tissue, as well as circulating DPP-4, correlated with adipocyte size and adipose tissue inflammation expressed as the percentage of macrophages in VAT.[38] *Ex vivo* studies showed that ^{18}F-FDG uptake is associated with macrophage infiltration in, for example, carotid plaques.[39,40] This combination of findings suggests the possibility that DPP-4 inhibition affects ^{18}F-FDG uptake in VAT. However, such a hypothesis is limited to the assumption that ^{18}F-FDG uptake in VAT is correlated with macrophage infiltration because it is observed in carotid plaques. Future studies on the association between ^{18}F-FDG uptake in VAT and macrophage infiltration in adipocytes are therefore recommended. Such an association could be investigated by collecting VAT during open abdominal surgery and investigating the feasibility of using ^{18}F-FDG for *ex vivo* imaging of adipose tissue. The results from µPET imaging of adipose tissue should be compared with the results of histology studies of macrophage infiltration and adipocyte size.

Cardiovascular risk in early type 2 diabetes

Over the past decade, it has become clear that not only high blood pressure and dyslipidemia but also inflammation are key contributors to the pathogenesis of atherosclerosis.[41-43] The development of an atherosclerotic lesion starts with endothelial cell dysfunction and activation of the endothelial monolayer, including adhesion of blood leukocytes into the intima resulting in local vascular inflammation.[41,42] Endothelial cell dysfunction and inelastic arteries indicate vascular dysfunction. Vascular function can be assessed as aortic PWV, which is considered the gold-standard for the non-invasive assessment of arterial stiffness. Small glycemic disturbances already display vascular dysfunction.[44,45] Furthermore, in type 2 diabetes several disturbances in the innate and adaptive immune system result in a low-grade chronic inflammatory state.[46-48] Recently, arterial ^{18}F-FDG uptake has been recognized as a surrogate of subclinical vascular inflammation.[49] Moreover, with advanced vascular imaging one can detect a person's state of vulnerability to CV risk. The state of vulnerability is probably more important than an individual plaque for assessment of CV risk. For example, determining the state of vulnerability to CV risk by measuring widespread vascular inflammation can be of clinical value. Therefore, the relationship between arterial ^{18}F-FDG uptake (mean of 10 arterial points of measurement) and aortic PWV was investigated in Chapter 5.

We found that in people with early type 2 diabetes (RELEASE participants) subclinical vascular inflammation is associated with vascular function, suggesting that inflammation is involved in arterial stiffness in early type 2 diabetes. However, we cannot state that this is a diabetes related effect. In a healthy non-diabetic population or for instance another population at high CV risk it may also be associated. The cross-sectional nature of the study described in Chapter 5 did not enable us to determine whether arterial stiffness is a cause or consequence of vascular inflammation. Further studies are required to address this. It

is possible that neither of them are causal and that a common denominator is involved, causing both arterial stiffness and vascular inflammation. Such a common denominator could be endothelial dysfunction.[50,51] Endothelial dysfunction has been implicated as a common pathogenic mechanism of type 2 diabetes and atherosclerosis. Circulating endothelial progenitor cells (EPCs) are used as a marker to assess endothelial function. EPCs facilitate endogenous repair mechanisms and are derived from hematopoietic stem cells. It is known that EPC count and its function are greatly impaired in patients with diabetes, insulin resistance and CVD.[52] Therefore, future investigations should measure EPCs and investigate their relationship with arterial stiffness and vascular inflammation, as well as the effects of antidiabetic drugs on EPCs.

Angiogenic T cells

In 2007, Hur *et al.* reported for the first time a subpopulation of T cells that constitute the central cell cluster of EPC colonies, assigned as angiogenic T cells (Tang).[53] These cells enhance the differentiation of early EPCs and promote neovascularization and endothelial repair. Tang cells possibly secrete proangiogenic cytokines, such as vascular endothelial growth factor, interleuking-8 and matrix metalloproteinases, promoting neovasculization.[53]

Recently, it was shown that Tang cells are strongly decreased in RA patients, compared with healthy controls, and negatively associated with disease activity in RA patients.[54] In hypertensive patients with cerebral small vessel disease (CSVD) the Tang cell count was significantly lower than in hypertensive patients without CSVD. This suggests a role for Tang cells in vascular disease.[55] So far, however, Tang cells has never been investigated in people with type 2 diabetes.

Tang cells are characterized by the co-expression of CD31 (platelet endothelial cell adhesion molecule) and CXCR4 (receptor for stromal cell-derived factor-1α [SDF-1 α]). In a clinical trial of 4 weeks in people with type 2 diabetes it was demonstrated that DPP-4 inhibition with sitagliptin led to an increase of EPCs, possibly mediated by SDF-1α.[56,57] Another study showed that DPP-4 inhibition reverses Tang cells dysfunction *in vitro* and improves angiogenesis *in vivo*.[58] Since CXCR4 is the receptor for SDF-1α, we hypothesize that DPP-4 activity may not only influence EPCs through SDF-1α, but also have an effect on Tang cells. Taking into account that DPP-4 activity in people with type 2 diabetes is increased when compared to non-diabetics, one might speculate that Tang cells constitutes a link between type 2 diabetes and CV risk. Moreover, DPP-4 inhibition may increase Tang cells. However, there have been no controlled studies to investigate Tang cell count and treatment effects of DPP-4 inhibition on Tang cell count in type 2 diabetes. The participants in the RELEASE study were treated with a DPP-4-inhibitor. Therefore, we recommend testing the hypothesis of Tang cell count in type 2 diabetes in the RELEASE participants. Furthermore, as characterization of the Tang cells can be difficult and depend on gating strategy to identify

the Tang cells cq EPC subpopulation, we strongly advise to include a healthy control group for comparison of the Tang cell count.

Interestingly, the chemokine CXCR4 is also found to be expressed in many human cancers and associated with tumor aggressiveness.[59] Therefore, CXCR4 is a target for molecular imaging in cancer research.[59,60] However, as expression of CXCR4 may also play a role in CVD, the tracers might also be of interest for CV risk assessment. In addition, it was recently shown that PET imaging with ^{68}Ga-pentixafor identified CXCR4 upregulation after acute myocardial infarction in humans.[61] However, future studies testing the usefulness of targeted CXCR4 imaging for CV risk assessment are needed before translating it to the clinical setting. Nevertheless, a randomized clinical trial to test treatment effects of a DPP-4 inhibitor on a ^{68}Ga-pentixafor-PET/CT scan should be undertaken to increase insight into the relationship between DPP-4 and atherosclerosis.

AGEs and DPP-4

AGEs are proteins or lipids that have become glycated after exposure to sugars, and they occur normally in the process of aging. However, as AGEs are produced extensively during hyperglycemia, they are a well-known consequence of chronic hyperglycemia. AGEs have unfavorable CV effects as a result of AGEs themselves and their binding to RAGE (receptor for AGE), which generates oxidative stress and inflammation. Hence, AGEs are associated with CVD and CVD mortality in people with type 2 diabetes.[62,63] Assessment of AGEs can easily be non-invasively performed with the AGE Reader™. The AGE Reader™ assesses AGEs in the skin utilizing the fluorescent properties of different AGEs; this is therefore referred to as skin autofluorescence (SAF).[64] In the RELEASE participants, SAF was positively associated with the extent of arterial calcification but not with arterial ^{18}F-FDG uptake and PWV (as presented in an EASD abstract 2015).[65] This finding indicates that AGEs are associated more with late stages of atherosclerosis than with early stage atherosclerosis.

Interestingly, levels of AGEs are independently correlated with circulating levels of DPP-4 in humans.[66] In addition, a recent extensive review demonstrated that there is crosstalk between the AGEs-RAGE axis and DPP-4 in the development and progression of CV complications.[67] The exact interplay between the AGEs-RAGE axis and DPP-4 is beyond the scope of this discussion. However, it is important to note that it has been suggested that serum AGEs levels may be clinical biomarkers to predict which people with type 2 diabetes will respond less to treatment with DPP-4 inhibitors.[67]

Favorable vascular effects of lingaliptin

The results of the RELEASE trial, described in Chapters 6 and 7, confirm the hypothesis that linagliptin may have favorable vascular effects. Treatment with linagliptin decreased PWV and arterial ^{18}F-FDG uptake, as compared with the placebo.[68,69] The underlying mechanism behind the favorable vascular effects of linagliptin remains unclear. DPP-4, as was pointed

out in the introduction to this thesis, has recently been recognized as a link between type 2 diabetes and CV risk. In brief, DPP-4 activity is increased during hyperglycemia and is known to cleave to many peptides, which influence the CV system. Furthermore, DPP-4 interacts with the immune system. Consequently, there are different mechanisms which could lead to favorable vascular effects of DPP-4 inhibition. Thus far we have already mentioned a few pathways of interest and suggested directions for further research. First, one pathway could be due to an increase of SDF-1α and the associated EPCs and possibly Tang cells. Second, since DPP-4 itself is also recognized as an adipokine, the metabolic activity of VAT could also influence the release of cytokines influencing the vascular system. Third, the interaction of DPP-4 with the AGE-RAGEs axis is another pathway of interaction with the vascular system. Fourth, upregulating the peptide GLP-1 results in increased insulin secretion (glycemic control); GLP-1 also has extra-glycemic effects, associated with favorable CV effects. So far, we have stated only a few potential mechanisms. It is clear that other mechanisms may also take place, probably also mechanisms that are yet to be discovered. In general we would suggest that the favorable vascular effects observed in the RELEASE study are a result of a combination of different mechanisms, or as Aristotle once said, "*The whole is greater than the sum of its parts*".

Clinical endpoint trials and clinical practice

The favorable vascular results observed in the RELEASE study differ from the results observed in the three published DPP-4 inhibitor outcome studies (EXAMINE[70], SAVOR[71], TECOS[72]), which showed no clear CV benefit. However, these trials included people with type 2 diabetes who also had CVD or were at high-risk of CVD, and who were using multiple glucose lowering drugs, including insulin. Therefore, their external validity is low compared with that of the RELEASE study population. Furthermore, in line with the suggestion that levels of AGEs predict who will respond to treatment with DPP-4 inhibitors, it is possible to speculate that the relationship between AGEs and DPP-4 could be a possible explanation for the lack of CV benefit in the outcome studies. If this suggestion is true, it may have an important implication: that the anti-atherogenic mechanisms of DPP-4 inhibitors can only take place early in the course of the disease of diabetes, before the occurrence of accelerated levels of AGEs and advanced atherosclerosis.

PWV and arterial ^{18}F-FDG uptake are surrogate endpoints. Therefore, it is unclear whether treatment with linagliptin will also result in a significant reduction of CV events in people with type 2 diabetes. A pooled analysis of 5847 patients on linagliptin compared to 3612 patients on comparator treatment (placebo or active) did not show a significant difference in primary CV outcomes (13.4 events per 100 patient-years versus 18.9 events[73]), while another meta-analysis showed significantly lower risk of CV events on linagliptin treatment.[74] In addition, two large endpoint trials on the CV safety of the DPP-4 inhibitor linagliptin [CAROLINA[75], CARMELINA[76]] are still ongoing and are expected to be completed

by 2018. These trials will provide more definitive evidence on the long-term CV effects of linaliptin. DPP-4 inhibitors differ in chemical structure and in the selectivity and potency of their enzyme inhibition;[77] therefore, the results of the CAROLINA[75] and CARMELINA[76] may differ from previous outcome studies. Furthermore, the CAROLINA[75] included 6041 people with early type 2 diabetes, with a median type 2 diabetes duration of 6.2 years, mean HbA_{1c} 7.2%; 66% were on 1 and 24% on 2 glucose-lowering agents and 34.5% had had previous CV complications. Clearly, the study population of the CAROLINA[75] differs from that of the previous outcome studies and possibly the results will also differ.

Although the RELEASE study showed positive results for linagliptin regarding CV risk markers, the first line therapy in type 2 diabetes remains metformin. Metformin has low cost, proven safety record, stable weight and possible benefits on CV outcomes.[78] Linagliptin is also weight neutral and has possible benefits on CV, but its long term CV safety has to be proven and its cost exceeds that of metformin. In the RELEASE study, a placebo arm was included as a control group. In future research, an active comparator arm including metformin should be carried out to establish the favorable CV effects of linagliptin and compare these effects with the current best practice with metformin.

Overall, the RELEASE study increased our insight into the possible mechanism underlying the favorable vascular effects of linagliptin in people with early type 2 diabetes. Challenging at this moment is to investigate whether those favorable vascular effects exceed those of metformin. As metformin will probably remain the first line therapy, further research should be undertaken to investigate the vascular effects of linagliptin as second line therapy along with metformin. This information is warranted before practical implications, such as changing the decision-making process for glucose lowering, can be acknowledged.

Imaging for characterization of atherosclerosis

Studies identifying high-risk plaques started with conventional morphologic imaging such as intravascular ultrasound and CT angiography to identify lumen stenosis and calcification. Then the PET/CT scan was introduced, combining the principles of biological imaging with morphological imaging. This combination, the PET/CT scan, proved to be a useful, novel multimodal technology in oncology. However, soon after the introduction of PET/CT, an ^{18}F-FDG uptake in arteries was reported.[79] As this arterial ^{18}F-FDG uptake was strongly correlated with the age-related increased risk of atherosclerosis, this was the beginning of the use of ^{18}F-FDG-PET/CT to assess atherosclerosis.[7] It turned out that ^{18}F-FDG uptake was related to macrophage infiltration and CV risk factors.[39,80,81] In 2006 Tahare *et al.,* were the first to report the effect of an intervention (statin therapy and diet versus diet alone) on arterial ^{18}F-FDG uptake on a ^{18}F-FDG-PET/CT scan.[82]

The use of a ^{18}F-FDG-PET/CT scan is nowadays an attractive instrumentation for characterization of atherosclerosis. Consequently, there are a great variety of imaging protocols and parameters used to investigate atherosclerosis, which limits the ability to

compare results between studies. Furthermore, the ability to investigate threshold and target values of arterial ^{18}F-FDG uptake on ^{18}F-FDG-PET/CT scans to reduce CV risk are therefore also limited. However, to overcome this challenge, in 2016 the Cardiovascular Committee of the European Association of Nuclear Medicine (EANM) published a position paper on PET imaging of atherosclerosis.[49] The goal of the EANM position paper was to harmonize imaging protocols regarding technical aspects such as ^{18}F-FDG dose and timing after injection, reconstruction protocols and parameters for quantification of tracer uptake. Clearly, in future studies we will take these EANM recommendations into account. For example, in the RELEASE study, a ^{18}F-FDG-PET/CT scan was performed 60 minutes after injection, as is the standard in oncology settings. However, for atherosclerosis PET imaging within an interval of 120 minutes is recommended.[49] Another important point for future trials is the sample size; an appropriate sample size is particularly important due to the radiation burden of PET/CT imaging. Recently van der Vlak *et al.* estimated, for arterial ^{18}F-FDG uptake in the carotid artery and aorta, sample sizes for vascular intervention studies ranging from 12 to 248.[16] This study can be helpful for future trials.

Since the introduction of PET/CT there has been a growing interest in the combination of PET/MRI. MRI is superior to CT for tissue analysis in some clinical situations, and is without radiation exposure. Therefore PET/MRI has emerged as an attractive and promising technique for several applications, including characterization of atherosclerosis. However, it can be quite challenging to develop a methodological strategy to optimize PET/MRI techniques.[83,84] Currently, no MRI acquisition protocols dedicated to vascular imaging are available. Although PET/MRI is mentioned as the most promising approach to identify vulnerable plaques as well as the heterogeneity of arterial inflammation, dedicated innovative imaging protocols are needed before PET/MRI can be introduced into clinical practice.

^{18}F-FDG is the most commonly used tracer for PET imaging, but other tracers are available and under development for use in atherosclerosis imaging.[85] This is needed, as the uptake of ^{18}F-FDG is hampered by obstacles. For instance the uptake of ^{18}F-FDG is in competition with glucose via the glucose transporter protein (GLUT), which is the most important pathway for glucose and ^{18}F-FDG to enter human cells.[86] To overcome this problem in imaging for diabetes, it is advised to correct for pre-scan glucose.[49] Furthermore, coronary imaging with ^{18}F-FDG is unreliable because of myocardial spillover.[87] One promising tracer is ^{68}Ga-DOTATATE with a high-specificity binding affinity for somatostatin receptor subtype-2 (SST_2).[88] SST_2 is expressed on activated macrophages and is therefore useful as a tracer for vascular inflammation. A recent prospective clinical study, VISION [Vascular Inflammation Imaging Using Somatostatin Receptor Positron Emission Tomography], evaluated ^{68}Ga-DOTATATE PET for coronary, carotid, and aortic inflammation in people with CVD and concluded that ^{68}Ga-DOTATATE as a novel marker of atherosclerotic inflammation was superior to ^{18}F-FDG.[89]

CONCLUSION

In this thesis we have investigated different clinical applications of vascular imaging. The identification of the high-risk atherosclerotic plaque is, with increasing vascular imaging modalities, in the spotlight. Another state of CV risk is the amount of VAT. We demonstrated a reliable automated method for the analysis of VAT, using a ^{18}F-FDG PET/CT scan. This method should be used in further investigations.

People with type 2 diabetes suffer from a seriously increased risk of CVD. Multimodal imaging with PET/CT provides greater insight into the interrelation of different CV markers in type 2 diabetes and may be useful for determining targets for treatment to reduce CV risk.[90] In addition, anti-diabetic drugs with the dual effect of lowering blood glucose levels and enhancing favorable vascular effects are preferred for treatment of type 2 diabetes. In the RELEASE trial we found that linagliptin monotherapy in early type 2 diabetes reduced PWV and arterial ^{18}F-FDG uptake, both markers of subclinical atherosclerosis.[68,69] This thesis has contributed evidence for the favorable vascular effects of linagliptin on subclinical atherosclerosis; however the challenge for future trials is to investigate whether those favorable vascular effects will translate into a significant reduction in CV events. Finally, with novel imaging modalities and new specific radiopharmaceutical tracers, the challenge for vascular imaging will be to translate these possibilities for use in personalized medicine.

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